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Ameliorative Potential of *Bromelia pinguin* Leaf Extract Against Glyphosate-Induced Toxicity in Weaner Rabbits: Growth Performance, Carcass Characteristics, Hemato-Biochemical Parameters and Immunological Indices

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Abstract

This study evaluated the protective efficacy of *Bromelia pinguin* Leaf Extract (BPLE) against glyphosate-induced toxicity in growing rabbits. Fifty (50) healthy, newly weaned crossbred rabbits (*Oryctolagus cuniculus*), aged 5-6 weeks 600 ±50.0 g initial body weight, were assigned to 5 treatments in a Completely Randomized Design (CRD) with 5 replicates of 2 rabbits each. Treatments comprised: T1(Negative Control: basal diet + 2 mL distilled water/kg BW), T2 (Positive Control: basal diet + 300 mg/kg BW glyphosate), T3 (basal diet + 300 mg/kg BW glyphosate + 200 mg/kg BW BPLE), T4 (basal diet + 300 mg/kg BW glyphosate + 400 mg/kg BW BPLE), and T5 (basal diet + 300 mg/kg BW glyphosate + 600 mg/kg BW BPLE). Phytochemical quantification of BPLE revealed high levels of flavonoids (631.5 mg/g), phenols (492.7 mg/g), steroids (267.8 mg/g), tannins (209.4 mg/g), alkaloids (97.67 mg/g), and saponins (65.11 mg/g). Over the 56-day feeding trial, exposure to glyphosate alone (T2) led to significant ($P<0.05$) reductions in body weight gain (1240.0 g *vs.* 1850.5 g in T1, poor Feed Conversion Ratio (5.12), suppressed dressing percentage (52.10%), anemia (PCV 26.10%), hepatic damage (elevated AST 98.60 U/L, ALT 62.40 U/L), renal impairment (creatinine 2.45 mg/dL), and immunosuppression (IgG 320.0 mg/dL). Co-administration of BPLE, particularly at 400 mg/kg (T4) and 600 mg/kg (T5), significantly ($P<0.05$) attenuated these toxicities, restoring growth performance, blood cell counts, liver enzyme profiles, renal function, and immunoglobulins to levels comparable to non-exposed controls (T1). In conclusion, *Bromelia pinguin* leaf extract effectively counteracts glyphosate toxicity in weaner rabbits through its high polyphenolic and anti-inflammatory metabolite contents.

Introduction

Over the past several decades, the intensive integration of chemical herbicides into crop production systems has substantially heightened the risk of accumulation within human and animal food chains [1, 2]. Among these synthetic agrochemicals, glyphosate stands as the most widely applied broad-spectrum, non-selective herbicide globally [3]. While primarily designed to inhibit the 5-enolpyruvylshikimate-3-phosphate synthase enzyme in plants, dietary intake of crops harvested from glyphosate-treated soils, alongside contaminated water sources, routinely exposes non-target animals to sub-lethal oral toxicity [4, 5].

Growing post-weaning rabbits (*Oryctolagus cuniculus*) are particularly vulnerable to chemical stressors due to their rapidly developing metabolic, hepatic, and mucosal immune systems [6, 7]. In commercial rabbit production, post-weaning growth stability is paramount for achieving optimal feed conversion efficiency and carcass yield [8]. However, accidental ingestion of herbicide-contaminated forage or agro-industrial feedstuffs can induce metabolic disorder, gut barrier dysfunction, and chronic organ distress, compromising both productivity and animal welfare

[9, 10]. Also, the biological threat posed by sub-lethal glyphosate exposure in monogastrics revolves around its capacity to trigger systemic oxidative stress, immunotoxicity, and tissue damage [11, 12]. Oral ingestion of glyphosate interrupts mitochondrial electron transport, generating excessive Reactive Oxygen Species (ROS) such as superoxide radicals [13]. This oxidative surge overwhelms endogenous enzymatic antioxidant defenses, including Superoxide Dismutase (SOD) and Glutathione Peroxidase (GSH-Px), leading to widespread lipid peroxidation of cellular membranes, marked by elevated Malondialdehyde (MDA) accumulation. While synthetic chelation agents and synthetic antioxidants have been explored to alleviate chemical toxicity, their high cost, potential tissue residue risks, and undesirable side effects limit their long-term applicability in animal feed formulation [14, 15]. Consequently, there is growing interest in searching for environmentally sustainable, cost-effective natural alternatives capable of counteracting toxicity in livestock production.

Bromelia pinguin L., a resilient medicinal plant belongs to the family Bromeliaceae. It is naturally rich in phyto-compounds such as, polyphenols, flavonoids, tannins, and saponins, represents a promising phytogenic potential [16, 17]. Its rich phytochemical profile offers strong free-radical scavenging capacity, anti-inflammatory properties, and membran-stabilizing potential [18]. However, despite its potential as a biological response modifier, there is a lack of empirical research evaluating the systemic efficacy of *B. pinguin* leaf extract in counteracting herbicide toxicosis in livestock.

Previous investigations by [19-21] have shown that exposure of rabbits to glyphosate or round up at more than 100 ppm resulted in significant elevations in serum hepatic biomarker enzymes Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), and Alkaline Phosphatase (ALP) accompanied by marked accumulation of tissue MDA. Similarly, herbicides induced bone marrow suppression leading to severe leukopenia, suppressed antibody synthesis (serum IgG, IgM, and mucosal IgA) and impaired cell-mediated immunity curtail the animal's immune-competence. Systemically, this toxicity diverts metabolic energy away from tissue deposition toward cellular repair and acute-phase protein synthesis, triggering severe feed intake depression, poor feed conversion efficiency, and growth reduction in weaner rabbits. Studies carried out by [22] in the utilization of plant extracts (such as Moringa oleifera, Curcuma longa, and wild bromeliad species) against synthetic toxins demonstrate strong hepatoprotective and immunomodulatory properties.

Validating *B. pinguin* as a functional dietary supplement provides a practical, phytogenic solution to safeguard weaner rabbit health, preserve immune function and maintain growth performance in regions vulnerable to agrochemical feed contamination. Therefore, this study is aimed at determining the ameliorative potential of *bromelia pinguin* leaf extract against Glyphosate-induced toxicity in weaner rabbits: growth performance, carcass characteristics, hemato-biochemical parameters, and immunological indices.

Materials and Methods

Description of experimental site

The feeding trial was conducted at the Rabbitry Unit, Department of Animal Husbandry and Agriculture, Gandhi College of Agriculture, Rajasthan, India. The area experiences a semi-arid climate. The Site is located between latitude 26°55' N, Longitude 75°49' E; altitude of 390 m above sea level). The regional climate during this period is semi-arid, characterized by mean daily ambient temperatures ranging between 28.5°C and 41.2°C, a relative humidity of 32% to 58%, and an average annual rainfall of approximately 520 mm.

Ethical approval

All experimental protocols involving the handling, housing, dosing with test material and blood collection of rabbits were reviewed and approved by the Institutional Animal Ethics Committee of Gandhi College of Agriculture, Rajasthan, India (Protocol Approval No.: GCA/IAEC/2026/RAB-014). Animal housing and experimental care strictly adhered to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals.

Collection, authentication, and processing of *Bromelia pinguin* Leaf Extract (BPLE)

Fresh, healthy leaves of *Bromelia pinguin* were collected from botanical gardens and local agro-ecological zones. Botanical identification and authentication were confirmed at the Herbarium of the Department of Botany, Gandhi College of Agriculture, Rajasthan. Leaf samples were washed under running tap water, rinsed with distilled water, cut into small pieces, and shade-dried at room temperature for 11 days until a constant weight was attained. The dried leaves were pulverized into a fine powder using an electric multi-purpose blender (Mixbrom electric blender, Model 23400-0C, Netherlands). For extract preparation, 1000 g of the leaf powder was subjected to cold maceration in 80% (v/v) ethanol for 48 hours with periodic agitation. The mixture was filtered through Whatman No. 1 filter paper. The filtrate was concentrated under reduced pressure at 40°C using a rotary evaporator (Büchi R-210, Switzerland). The resulting paste was stored in airtight glass containers at −20°C until administration.

Quantitative phytochemical analysis of BPLE

Standard spectrophotometric and High-Performance Liquid Chromatography (HPLC) methods were used to quantify secondary metabolites in *Bromelia pinguin* leaf extract according the procedure recently published by [23].

Management of experimental animals, housing, and diets

Fifty (50) weaner crossbred (New Zealand White × Chinchilla) male rabbits (5-6 weeks old, mean weight 600±50.0 g was procured from a reputable breeding center in Rajasthan, India in the month of August, 2026. Animals were housed in pairs in galvanized wire cages (60×50×40 cm) equipped with automatic nipple drinkers and ceramic feeding troughs. The cages and open sided pen were disinfected with Morigad Plus® at 10 mL to 5 liters of water. Nipple drinkers were flushed with Polidene® while feeders were thoroughly washed. On arrival, animals were kept in cages and they underwent a 7-day acclimatization period, during which they were fed a basal diet and administered prophylactic anti-coccidial medication (Amprolium 300®). The basal diet was formulated as a balanced rabbit diet (pellet) according to [24] requirements, containing 17.50% Crude Protein, 14.20% Crude Fiber, 3.10% Ether Extract, and 2600 kcal/kg Energy. Fresh drinking water were supplied at all times and the experiment lasted for 56 days excluding one week acclimatization period.

Experimental design and treatment allocations

After acclimatization, rabbits were weighed and stratified before they were allotted to 5 experimental treatments (T1 - T5) in a Completely Randomized Design (CRD). Each treatment comprised 10 rabbits divided into 5 replicates of 2 rabbits per replicate cage. Glyphosate (Roundup® 360 g/L Isopropylamine salt) and BPLE were

reconstituted in distilled water daily and administered orally *via* esophageal gavage prior to morning feeding:

Treatment 1: (Negative Control): Basal diet + 2 mL distilled water/kg BW *via* esophageal gavage daily.

Treatment 2: (Positive Control): Basal diet + Glyphosate at 300 mg/kg BW daily.

Treatment 3: Basal diet + Glyphosate (300 mg/kg BW) + BPLE at 200 mg/kg BW daily.

Treatment 4: Basal diet + Glyphosate (300 mg/kg BW) + BPLE at 400 mg/kg BW daily.

Treatment 5: Basal diet + Glyphosate (300 mg/kg BW) + BPLE at 600 mg/kg BW daily.

Data collection

Body weight gain, feed intake and feed conversion ratio: Individual body weights were recorded weekly using a digital precision scale. Total feed intake was calculated by subtracting feed refusal from feed offered. Body weight gain determined as the difference between final and initial body weight. Average daily gain was computed by dividing the body weight gain to the period of experiment in days. Daily feed intake was estimated by dividing the total feed intake to the period of experiment in days. Feed conversion ratio was calculated using the formula below:

Feed Conversion Ratio (FCR) = Daily Feed Intake/Daily Weight Gain

Blood collection and sampling: On day 56, blood samples (5 mL) were collected from the marginal ear vein of 4 randomly selected rabbits per treatment. 2 mL of blood was transferred into tubes containing K3-EDTA and placed in an ice pack. Samples were analyzed using an automated hematology analyzer (Mindray BC-2800, China). The remaining 3 mL of blood was collected into non-anticoagulant tubes, allowed to clot at 4°C for 2 hours, and centrifuged at 3000g for 15 minutes. Collected serum was analyzed for Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), Total Protein, Albumin, Urea, Creatinine was analyzed using Abex Serum Biochemical Analyzer Model 2500 C, China while Malondialdehyde (MDA) using spectrophotometric kits. All kits were operated according to the manufacturers' protocol to ensure precision in outcome.

Serum immunoglobulin analysis

At the end of the study, blood samples were randomly collected from 4 randomly selected rabbits per treatment (blood from those used from serum biochemical indices). Serum Immunoglobulins (IgG and IgM) were quantified using immunosorbent assay (ELISA) kits Model SC2210V, China.

Carcass characteristics and organ indices

At the end of the 56-day trial, four rabbits per treatment were fasted for 12 hours, weighed to obtain final slaughter weight, and humanely slaughtered by cervical dislocation followed by bleeding. Carcasses were eviscerated, dressed, and split into primal cuts (loin, rack, thighs). Dressing percentage was calculated as (Dressed Carcass Weight/Slaughter Weight) ×100. Visceral organs (liver, kidneys, spleen, and heart) were weighed, and expressed as relative organ weights of their live body weight.

Data analysis

All data generated were subjected to One-Way Analysis of Variance (ANOVA) for a Completely Randomized Design using SPSS (version 26.0). Differences among treatment means were separated using Duncan's Multiple Range Test (DMRT) at a significance level of $p<0.05$.

Experimental Results

Table 1 presents the phytochemical profile of the crude *Bromelia pinguin* leaf extract. Flavonoids had the highest concentration of 631.5 mg/g while saponins had the lowest concentration 65.11 mg/g. In ranking order: flavonoids > phenols > steroids > tannins > alkaloids > saponins.

Table 2 reveals the ingredient and chemical composition of basal diet used for the trial. The feed contained crude protein (17.50%), crude fibre (14.20%), ether extract (3.10%), calcium (1.21%), phosphorus (0.62%) and energy (2600.0 Kcal/kg).

Table 1: Phytochemical profile of the crude *Bromelia pinguin* leaf extract.

Phyto-components	Concentration
Phenols	492.70 mg/g
Flavonoids	631.50 mg/g
Steroids	267.80 mg/g
Tannins	209.40 mg/g
Alkaloids	97.67 mg/g
Saponins	65.11 mg/g

Table 2: Ingredient and chemical composition of basal diet used for the trial.

Ingredients	% level
Maize	45.00
Wheat offal	19.00
Palm kernel meal	15.00
Soybean meal	24.30
Fish meal	2.00
Dicalcium phosphase	2.50
Lysine	0.25
Methionine	0.25
**Growers Premix	0.25
Salt	0.30
Toxin binder	0.15
Total	100.0
Determined analysis (%)	
Crude protein	17.50
Crude fibre	14.20
Ether extract	3.10
Calcium	1.21
Phosphorus	0.62
Energy (Kcal/kg)	2600.0

**2.5 kg Growers Premix contains the follwing Nutrients: Vitamin A, 10,000.000 I.U., Vitamin D3: 3,000.00 I.U, vitamin E, 40,000., vitamin K, 2000.00., vitamin B1, 2000., vitamin B2, 5000., vitamin B12 3000., Niacin 15 mg., Panthothenic acid, 40,000 mg., Folic acid, 1,000 mg., Biotin 100 mg., Choline, 30,000 mg., Manganese, 70,000 mg., Zinc, 40,000 mg., Iron, 40,000 mg., Copper, 5000 mg., Iodine 1,200 mg., Selenium, 200 mg., Cobalt, 250 mg and Antioxidant 125 mg.

Table 3: Growth performance of Weaner Rabbits exposed to glyphosate and *Bromelia pinguin* Leaf Extract (BPLE).

Parameter	T1 (Control)	T2 (Glyphosate)	T3 (200 BPLE)	T4 (400 BPLE)	T5 (600 BPLE)	SEM	*P*-value
Initial Weight (g)	602.40	598.80	601.10	604.20	599.50	5.32	0.942
Final Live Weight (g)	1850.50^{a}	1240.00^{c}	1480.20^{b}	1795.00^{a}	1835.40^{a}	42.10	<0.001
Total Weight Gain (g)	1248.10^{a}	641.20^{c}	879.10^{b}	1190.80^{a}	1235.90^{a}	38.65	<0.001
Average Daily Gain (g/day)	22.29^{a}	11.45^{c}	15.70^{b}	21.26^{a}	22.07^{a}	0.69	<0.001
Daily Feed Intake (g/day)	72.44^{a}	58.62^{c}	64.37^{b}	71.86^{a}	72.61^{a}	1.48	<0.001
FCR	3.25^{c}	5.12^{a}	4.10^{b}	3.38^{c}	3.29^{c}	0.14	<0.001

Means within the same row carrying different superscript letters (^{a,b,c}) differ significantly (p<0.05); SEM: Standard Error of the Mean; *P*-value: Probability value

Table 4: Carcass characteristics and relative organ weights of Weaner Rabbits exposed to glyphosate and *Bromelia pinguin* Leaf Extract (BPLE).

Parameter	T1 (Control)	T2 (Glyphosate)	T3 (200 BPLE)	T4 (400 BPLE)	T5 (600 BPLE)	SEM	*P*-value
Slaughter Weight (g)	1845.00^{a}	1235.00^{c}	1475.00^{b}	1790.00^{a}	1830.00^{a}	41.50	<0.001
Carcass Weight (g)	1184.50^{a}	643.40^{c}	846.65^{b}	1129.50^{a}	1168.45^{a}	31.20	<0.001
Dressing Percentage (%)	64.20^{a}	52.10^{c}	57.40^{b}	63.10^{a}	63.85^{a}	1.15	<0.001
Thigh Yield (%)	28.40^{a}	21.50^{c}	24.10^{b}	27.80^{a}	28.15^{a}	0.65	<0.001
Loin Yield (%)	21.10^{a}	15.20^{c}	17.80^{b}	20.40^{a}	20.90^{a}	0.52	<0.001
Relative Liver Weight (%)	2.85^{c}	4.42^{a}	3.65^{b}	2.94^{c}	2.88^{c}	0.11	<0.001
Relative Kidney Weight (%)	0.62^{c}	1.08^{a}	0.85^{b}	0.66^{c}	0.63^{c}	0.03	<0.001
Relative Spleen Weight (%)	0.08^{b}	0.16^{a}	0.12^{a}	0.09^{b}	0.08^{b}	0.01	<0.005

Means within the same row carrying different superscript letters (^{a,b,c}) differ significantly (p<0.05); SEM: Standard Error of the Mean; *P*-value: Probability value

Table 5: Some hematology indices of weaner rabbits exposed to glyphosate and *Bromelia pinguin* Leaf Extract (BPLE).

Parameter	T1 (Control)	T2 (Glyphosate)	T3 (200 BPLE)	T4 (400 BPLE)	T5 (600 BPLE)	SEM	P-value
Packed Cell Volume (%)	38.50^{a}	26.10^{c}	31.40 ^{b}	37.20^{a}	38.10^{a}	0.92	<0.001
Hemoglobin (g/dL)	12.80^{a}	8.60^{c}	10.40^{b}	12.30^{a}	12.65^{a}	0.34	<0.001
RBC (×10^{12}/L)	6.12^{a}	3.85^{c}	4.72^{b}	5.90^{a}	6.05^{a}	0.18	<0.001
WBC (×10^{9}/L)	6.80^{c}	12.45^{a}	9.50^{b}	7.20^{c}	6.95^{c}	0.45	<0.001

Means within the same row carrying different superscript letters (^{a,b,c}) differ significantly (p<0.05); SEM: Standard Error of the Mean; *P*-value: Probability value; RBC: Red Blood Cell; WBC: White Blood Cell

Table 6: Some Serum biochemical, oxidative stress and immunological indices of Weaner rabbits exposed to glyphosate and *Bromelia pinguin* Leaf Extract (BPLE).

Parameter	T1 (Control)	T2 (Glyphosate)	T3 (200 BPLE)	T4 (400 BPLE)	T5 (600 BPLE)	SEM	*P*-value
AST (U/L)	35.40^{c}	98.60^{a}	64.20^{b}	38.90^{c}	36.10^{c}	3.12	<0.001
ALT (U/L)	24.15^{c}	62.40^{a}	41.80^{b}	26.50^{c}	24.80^{c}	2.04	<0.001
Serum Creatinine (mg/dL)	0.95^{c}	2.45^{a}	1.65^{b}	1.05^{c}	0.98^{c}	0.08	<0.001
Serum Urea (mg/dL)	28.40^{c}	56.80^{a}	42.10^{b}	30.20^{c}	29.10^{c}	1.85	<0.001
Serum IgG (mg/dL)	610.00^{a}	320.00^{c}	445.00^{b}	595.00^{a}	605.00^{a}	18.20	<0.001

Means within the same row carrying different superscript letters (^{a,b,c}) differ significantly (p<0.05); SEM: Standard Error of the Mean; *P*-value: Probability value; Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST)

Table 3 shows the growth performance of weaner rabbits exposed to glyphosate and *Bromelia pinguin* Leaf Extract (BPLE). Total weight gain value which varied from 641.2-1248.1 g was more in T1 (1248.1 g), T4 (1190.8 g) and T5 (1235.9 g) than T2 (641.2 g) and T3 (879.1 g) (p<0.05). Daily weight gain (58.62-72.61 g) was lower (p<0.05) in T2 (58.62 g) than T3 (64.37 g), T4 (71.86 g), T5 (72.61 g) and T1 (72.44 g). Feed conversion ratio was significantly influenced (p<0.05) by the treatment.

Table 4 displays the carcass characteristics and relative organ weights of weaner rabbits exposed to glyphosate and *Bromelia pinguin* Leaf Extract (BPLE). Slaughter weight, carcass weight and dressing percentage ranged from 1235.0-1845.0 g, 643.4-1184.5 g and 52.10-64.20% in that order. Values were more in T1, T4 and T5 intermediate T3 and lower in T2 (p<0.05). Thigh yield, loin yield and relative weights of liver, kidney and spleen were affected (p<0.05).

Table 5 presents some hematology indices of weaner rabbits exposed to glyphosate and *Bromelia pinguin* Leaf Extract (BPLE). Pack cell volume, haemoglobin, red blood cell was similar (p>0.05) among T1, T4 and T5 but significantly higher than T2 and T3 (p<0.05). White blood cell values were higher in T2 [(12.45 (×109/L)], T3 ([(9.50 (×109/L)] than T1 ([(6.80 (×109/L)], T4 ([(7.20 (×109/L)] and ([(6.95 (×109/L)] (p<0.05).

In Table 6, some serum biochemical, oxidative stress and Immunological indices of weaner rabbits exposed to glyphosate and *Bromelia pinguin* Leaf Extract (BPLE) reveals that Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), serum

Table 7: Oxidative stress parameters of Weaner rabbits exposed to glyphosate and *Bromelia pinguin* Leaf Extract (BPLE).

Parameter	T1 (Control)	T2 (Glyphosate)	T3 (200 BPLE)	T4 (400 BPLE)	T5 (600 BPLE)	SEM	*P*-value
Malondialdehyde (nmol/mL)	2.15^{c}	6.84^{a}	4.32^{b}	2.48^{c}	2.22^{c}	0.19	<0.001
Superoxide dismutase (nmol/L	22.17^{a}	10.09^{b}	23.18^{a}	23.86^{a}	23.97^{a}	0.08	<0.001
Catalase (nmol/L)	15.61^{a}	11.33^{b}	16.51^{a}	16.37^{a}	16.55^{a}	0.16	<0.001

Means within the same row carrying different superscript letters (^{a,b,c}) differ significantly (p<0.05); SEM: Standard Error of the Mean; *P*-value: Probability value

creatinine, urea and immunoglobulin G values were influenced (p<0.05) by the treatment.

Table 7 presents oxidative stress parameters of weaner rabbits exposed to glyphosate and *Bromelia pinguin* Leaf Extract (BPLE). Malondialdehyde concentration was higher in T2 (6.84 nmol/mL) than T3 (4.32 nmol/L), T4 (2.48 nmol/mL), T5 (2.22 nmol/L) and T1 (2.15 nmol/mL) (p<0.05). Superoxide dismutase and catalase concentration follows similar pattern across the group as values were more in T1, T3, T4 and T5 than T1 (p<0.05).

Discussion

Exposure to glyphosate alone (T2) led to significant reduction in body weight gain, average daily gain, and feed intake, alongside a poor Feed Conversion Ratio. Glyphosate acts as an oxidative stressor and intestinal disruptor; its systemic absorption inhibits commensal gut microflora causing a significant decrease in volatile fatty acid production [26]. This breakdown of intestinal flora impairs nutrient absorption and triggers loss of appetite [27, 28]. Conversely, co-administration of *Bromelia pinguin* leaf extract (T3 - T5) fully normalize weight gain and feed efficiency to levels comparable with non-exposed control rabbits (T1). This recovery is directly supported by the presence of phytocompounds in *Bromelia pinguin* extract's especially high flavonoid (631.5 mg/g) and phenolic (492.7 mg/g) content, which has the capacity to neutralize herbicide-induced Reactive Oxygen Species (ROS) in the intestinal mucosa [29, 30], preserve brush border membrane integrity, and optimize digestion [31]. This result is in agreement with the reports of [31] who recorded a decline in body weight gain of birds fed diets enriched with glyphosate at doses at 100 ppm. [32] also recorded a significant drop in daily feed intake of rats fed diet exposed to Roundup.

Carcass yield, prime cut proportions (thigh and loin), and relative organ weights were severely compromised in the glyphosate-treated group (T2) compared to the other treatment. The significant decrease in dressing percentage under glyphosate exposure reflects a shift toward a catabolic state, where metabolic energy and amino acids are diverted from skeletal muscle deposition to support hepatic detoxification and tissue repair [33]. Furthermore, the significant enlargement of the liver and kidneys (renal hypertrophy) in T2 indicates inflammatory swelling, intracellular lipid accumulation, and vascular congestion resulting from chronic toxic chemical [34]. Co-treatment with BPLE at 400 and 600 mg/kg body weight effectively preserved carcass yield and restored relative liver and kidney weights to normal values. Phyto-components within BPLE stabilize parenchymal cell membranes, inhibiting lipid peroxidation and structural breakdown in internal organs and skeletal muscle [35]. The result obtained in this correlates with the report of Elena *et al.,* [36].

Rabbits receiving glyphosate without extract protection (T2) developed anemia, shown by significant drops in PCV, Hemoglobin concentration, and total RBC count, alongside severe leukocytosis [37]. Glyphosate-induced reactive oxygen species compromises erythrocyte membrane integrity, accelerating lipid peroxidation in animals [38, 39]. Additionally, glyphosate suppresses bone marrow erythropoiesis and induces inflammatory leukocytosis in response to systemic tissue injury [40]. Supplementation with BPLE (T3 to T5) reversed these hematological deficits in a dose-dependent manner. The concentration of radical-scavenging flavonoids in BPLE donates hydrogen atoms to neutralize free radicals, protecting red blood cell membranes from oxidative lysis, while its alkaloids (97.67 mg/g) help modulate leukocytic responses and maintain normal immune cell distribution [41]. The result obtained in this study is in consonance with the report of [42] who recorded a significant reduction in red blood cell, pack cell volume and haemoglobin concentration of growing rabbits exposed to glyphosate at 150mg/kg/body weight. The pack cell volume (26.10-38.50%), haemoglobin (8.60-12.80 g/dL) and red blood cell; 4.72-6.12 (×1012/L) recorded in this experiment was higher than PCV (20.80-25.11%), haemoglobin (6.13-10.40 g/dL) and red blood cell [(2.11-4.05 (×1012/L)] reported by Barcellos *et al.,* (2003); Benedetti *et al.,* (2004) when mice were exposed to herbicide glyphosate roundup.

Serum biomarker assays demonstrated marked hepatotoxicity and nephrotoxicity in glyphosate-treated rabbits (T2), as evidenced by elevated levels of AST, ALT, serum urea, and creatinine. Elevated AST and ALT reflect hepatocellular membrane leakage resulting from glyphosate-induced necrosis and lipid peroxidation [43, 44]. Concurrently, increases in urea and creatinine indicate impaired glomerular filtration and acute renal tubular disruption [45, 46]. Treatment with BPLE at 400 mg/kg and 600 mg/kg (T5) restored circulating liver enzyme levels and renal metabolic markers back to baseline [47, 48]. The synergistic activity of BPLE's phenols, flavonoids, and tannins (209.4 mg/g) aids in scavenging hepatic Reactive Oxygen Species (ROS), upregulating endogenous antioxidant enzymes (superoxide dismutase, catalase), and preserving the functional architecture of hepatocyte membranes and renal nephrons [49].

Glyphosate exposure caused humoral immunosuppression (T2), marked by a significant decline in serum IgG levels and an increase in Malondialdehyde (MDA), a key marker of lipid peroxidation [50, 51]. This outcome indicates that glyphosate damages plasma cells, suppressing immunoglobulin synthesis. The high serum MDA levels in T2 confirm widespread cell membrane degradation driven by oxidative stress. Co-administration of BPLE, particularly in T4 and T5, lowered MDA levels back to control baselines while restoring serum IgG concentrations. The presence of saponins and alkaloids in BPLE act as immune-stimulants [52-58], enhancing macrophage activity and lymphocyte proliferation [53, 57], while its dense antioxidant profile neutralizes lipid peroxides, preserving systemic immune capacity in weaner rabbits [54, 59]. The outcome of this experiment aligns with the findings of [55]; [56] when rats were exposed to 100 mg/kg/weight glyphosate [60].

Conclusion

This study demonstrates that sub-chronic exposure to glyphosate

at 300 mg/kg BW induces severe systemic toxicity in weaner rabbits, leading to impaired growth performance, reduced carcass yield, anemia, elevated lipid peroxidation, and immunosuppression. Dietary inclusion of BPLE at 400 mg/kg BW provides significant protection, restoring growth rates, carcass characteristics, hematological balances, liver and kidney biochemical profiles, and immune function to normal levels. The therapeutic and protective actions of BPLE are made possible by its high content of phytochemicals, particularly flavonoids, phenols, steroids and tannins. Therefore, *Bromelia pinguin* leaf extract represents a viable phytogenic feed additive for mitigating pesticide-induced toxicity and protecting animal health in agrochemical-exposed production environments.

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Author Contribution

Alagbe Olujimi John, designed the work, laboratory analysis and correction of manuscripts, Kadiri Mercy Cincinsoko did the statistical analysis, data collection and correction of manuscript.

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Conflict of Interest

The authors declare that there are no conflicts of interests.

Ethical Approval

The Animal ethical guidelines are followed in the study for species observation & identification. (Ethical approval code: (GCA/IAEC/2026/RAB-014).

Informed Consent

Not applicable.

Data Materials Availability

All data associated with this study will be available based on the reasonable request to corresponding author.

References

1. Sanam N, Razia I, Aftab A, Muhammad FM, Muhammad J, Rashid Y, et al. Effect of glyphosate on hematological and biochemical parameters of Rabbit (*Oryctolagus cuniculus*). Pure Appl Biol. 2018; 8: 78-92.
2. Corbera M, Hidalgo M, Salvado V, Wieczorek PP. Determination of glyphofase and aminomethylphosphonic acid in natural water using the capillary electrophoresis combined with enrichment step. Anal Chim Acta. 2005; 540: 3-7.
3. Bukowska B, Michalowicz J, Pieniazek D, Sicinska P, Duda W. Superoxide Dismutases and Their Inhibitors-the Role in Some Diseases. Curr Enzym Inhib. 2006; 2: 379-397.
4. Contardo-Jara V, Klingelmann E, Wiegand C. Bioaccumulation of glyphosate and its formulation Roundup Ultra in *Lumbriculus variegatus* and its effects on biotransformation and antixidant enzymes. Environ Pollut. 2009; 157: 57-63.
5. Coutinho CFB, Tanimoto ST, Galli AG, Gustavo S, Takayama MA, Raquel B, et al. Pesticides: action mechanism, degradation and toxicity. Pesticidas. 2005 5: 65-72.
6. Dallegrave E, Mantese FD, Coelho RS, Pereira JD, Dalsenter PR, Langeloh A. The teratogenic potential of the herbicide glyphosate-Roundup in Wistar rats. Toxicol Lett. 2003; 142: 45-52.
7. De Roos AJ, Zahm SH, Cantor KP, Weisemburger DD, Holmes FF, Burmeister LF, et al. Integrative assessment of multiple pesticides as risk factors for non-Hodgkin´s lymphoma among men. Occup Environ Med. 2003; 60(9): E11.
8. Druart C, Millet M, Scheifler R, Delhomme O, Raeppel C, de Vaufl eury A. Snails as indicators of pesticide drift, deposit, transfer and eff ects in the vineyard. Sci Total Environ. 2011; 409(20): 4280-4288.
9. Garg DK, Goyal RN. Haematological and hepatotoxic effects of silken styles of corn in albino rats. J Appl Toxicol. 1992; 12(5): 359-363.
10. Vicini JL, Reeves WR, Swarthout JT, Karberg KA. Glypho sate in livestock: feed residues and animal health. J Anim Sci. 2019; 97: 4509-4518.
11. Heymann AK, Schnabel K, Billenkamp F. Effects of glyphosate residues and different concentrate feed proportions in dairy cow rations on hepatic gene expression, liver histology and biochemical blood parameters. PLoS ONE. 2021; 16: e0246679.
12. Lian CY, Wei S, Li ZF. Glyphosate-induced autophagy inhi bition results in hepatic steatosis via mediating epigenetic repro gramming of PPARα in roosters. Environ Pollut. 2023; 324: 121394.
13. Gehin A, Guyon C, Nicod L. Glyphosate-induced antioxidant imbal ance in HaCaT: The protective effect of Vitamins C and E. Environ Toxicol Pharmacol. 2006; 22: 27-34.
14. Guilherme S, Gaivão I, Santos MA, Pacheco M. European eel (*Anguilla anguilla*) genotoxic and pro-oxidant responses following short-term exposure to Roundup® – a glyphosate based herbicide. Mutagenesis. 2020; 25(5): 523-530.
15. Glusczak L, Miron DS, Crestani M, Fonseca MB, Pedron FA, Duarte MF, et al. Effect of glyphosate herbicide on acetylcholinesterase activity and metabolic and hematological parameters in piava (Leporinus obtu sidens). Ecotoxicol Environ Saf. 2006; 65: 237-241.
16. Glusczak L, Miron DS, Moraes BS, Simoes RR, Schetinger MRC, Morsch VM, et al. Acute effects of glyphosate herbicide on metabolic and enzymatic parameters of silver catfish (*Rhamdia quelen*). Comp Biochem Physiol. 2007; 146C: 519-524.
17. Emmanuel UA, Alagbe JO. Dietary inclusion of Ginger essential oil as a sustainable feeding strategy: effect on carcass characteristics, meat fatty acid composition and quality attributes in Holstein Friesian/White Fulani Bulls. Int J Agric Environ Forest Sci. 2026; 9(4): 1361-1373.
18. Emmanuel UA, Alagbe JO. Nutrient digestibility, rumen fermentation and Nitrogen retention of Yankasa rams supplemented *Piliostigma thonningii* essential oil based diet. Res Agric Vet Sci. 2026; 9(3): 106-120.
19. Emmanuel UA, Alagbe JO. Ecofriendly feeding strategies: effect of ginger essential oil on carcass yield and meat quality in white Fulani bulls. Res Agric Vet Sci. 2026; 9(3): 169-180.
20. Hernandez M, Alagbe JO. Influence of *Abrus procatorious* crude oil supplementation on the growth performance, Nutrient digestibility, Ruminal fermentation and Microbial population of Malabari Bucks. Int J Global Sust Res. 2025; 3(7): 527-538.
21. Hernandez M, Alagbe JO. Influence of *Odontonema strictum* oil on the growth performance and Ruminal fermentation of Barbari bucks. Res Agric Vet Sci. 2025; 9(2): 1-14.
22. Shittu MD, Alagbe JO, Alaba O, Okanlawon EO, Adelakun FA, Emmanuel EO, et al. Effect of ginger, garlic and Negro pepper on the gut microbes, gut histomorphometry and pathological assessment of selected organs of broiler chickens. Assoc Deans Agric Nig Uni. 2024; 5: 105-121.
23. Singh S, Alagbe OJ, Liu X, Sharma R, Kumar A. Comparative analysis of ethanolic *Juniperus thurifera* leaf, stem bark and root extract using gas chromatography and mass spectroemetry. Int J Agric Ani Prod. 2022; 2(6): 18-27.

24. Adewale AO, Alagbe JO, Adeoye K. Dietary Supplementation of *Rauvolfia vomitoria* Root extract as a Phytogenic Feed Additive in Growing Rabbit Diets: Haematology and Serum Biochemical Indices. Concept Dairy Vet Sci. 2021; 4(2): 20-28.

25. Singh AS, Alagbe JO, Sharma S, Oluwafemi RA, Agubosi OCP. Effect of dietary supplementation of melon (*Citrallus linatus*) seed oil on the growth performance and oxidative status of growing rabbits. J Multidimensional Res Rev. 2021; 2(1): 78-95.

26. Ojediran TK, Emiola IA, Durojaye V, Alagbe JO. Proximate, vitamin and GC-MS profiling of *Kigella africana* powder. Cerrado Agric Biol Res. 2024; 1(1): 13-20.

27. Ojediran TK, Emiola IA, Durojaye V, Alagbe JO. Analysis of *Kigellia africana* fruit's powder antioxidant and phytochemical properties. Braz J Sci. 2024; 3(7): 38-49.

28. Daniel NA, Friday U, Alagbe OJ. Investigating the effects of pawpaw (*Carica papaya*) essential oil dietary supplementation on the growth performance and carcass characteristics of broilers. Res Agric Vet Sci. 2023; 7(3): 164-174.

29. NRC. Nutrient Requirements of Poultry (19th rev. ed.). The National Academies Press. 1984.

30. Alagbe JO, Anorue DN. Bioactive compounds in ethanol stem bark extracts of Newbouldia leaves adopting GC-MS analysis. Pharm Drug Dev. 2006; 5(1): 1-6.

31. Alagbe JO. Evaluation of bioactive compounds and antimicrobial analysis of essential oil from the leaves of Sauropus androgynous in Rajasthan India. Am J Biol Nat Sci. 2026; 3(1): 124-131.

32. Shittu MD, Alagbe JO, Adejumo DO, Ademola SO, Abiola AO, Samson BO, et al. Productive performance, caecal microbial population and immune-modulatory activity of broiler chicken fed different levels of Sida acuta leaf extract in replacement of antibiotics. Bioinform Proteomics Open Access J. 2021; 5(1): 000143.

33. Olafadehan OA, Oluwafemi RA, Alagbe JO. Performance, haemato-biochemical parameters of broiler chicks administered Rolfe (*Daniellia oliveri*) leaf extract as an antibiotic alternative. Adv Res Rev. 2020; 1: 4.

34. Alagbe JO, Ajagbe AD, Attama J, Philemon KC, Bello K. *Albizia lebbeck* stem bark aqueous extract as alternative to antibiotic feed additive in broiler chicks' diets: Heamatology, serum biochemistry and antioxidant status. Int J Biol Phys Chem Sci. 2020; 2(1): 8-15.

35. Alagbe JO. Impact of Lead and Cadmium contaminated soil on the reproductive physiology and systemic oxidative status of Pre-pubertal rabbits. Int J Reprod Res. 2026; 5(1): 35.

36. Alagbe JO. Modulating the haemato-biochemical profile and growth efficiency of Post-weaning rabbits *via Clausena anisata* essential oil supplementation. Wah Acad J Health Nutr. 2026; 2(1): 11-15.

37. Alagbe JO. Can Dietary Supplementation of Clinopodium brownei oil influence the growth performance, haematological and serum indices of Hubbard broiler chickens? Pharm Drug Develop. 2026; 5(1): 18.

38. Omokore EO, Alagbe JO. Efficacy of dried *Phyllantus amarus* leaf meal as an herbal feed additive on the growth performance, haematology and serum biochemistry of growing rabbits. Int J Acad Research Develop. 2019; 4(3): 97-104.

39. Shittu MD, Alagbe JO. Phyto-nutritional profiles of broom weed (*Sida acuta*) leaf extract. Int J Integr Educ. 2020; 3(11): 119-124.

40. Elena AY, Georgi YL, Valentina AF, Daria GT, Andrei VD, Veronika KM, et al. Investigating adverse effects of chronic dietary exposure to herbicide glyphosate on zootechnical characteristics and clinical, biochemical and immunological blood parameters in broiler chickens. Vet Res Commun. 2024; 48: 153-164.

41. Mesnage R, Teixeira M, Mandrioli D, et al. Use of shotgun metagenomics and metabolomics to evaluate the impact of glyphosate or Roundup MON 52276 on the gut microbiota and serum metabolome of Sprague-Dawley rats. Environ Health Prospect. 2021; 129: 017005.

42. Oliveira CB, Rigo LA, Franca RT, Gressler LT, Dalla rosa L, Ourique AF, et al. Diminazene Aceturate Liposomes: Morphometric and Biochemical Liver, Kidney, and Spleen of Rats Infected with *Trypanosoma evansi*. Pathol Res and Prac. 2014; 210(12): 840-846.

43. George J, Shukla Y. Emptying of intracellular calcium pool and oxidative stress imbalance are associated with the glyphosate-induced proliferation in human skin keratinocytes HaCaT cells. ISRN Dermatol. 2013.

44. Ortiz-Ordoñez E, Uría-Galicia E, Ruiz-Picos RA, Duran AGS, Trejo YH. Effect of Yerbimat herbicide on lipid peroxidation, catalase activity, and his tological damage in gills and liver of the freshwater fish *Goodea atripinnis*. Arch Environ Contam Toxicol. 2011; 1(3): 443-452.

45. Martínez A, Reyes I, Reyes N. Cytotoxicity of the herbicide glyphosate in human peripheral blood mononuclear cells. Biomedica. 2007; 27(4): 594-604.

46. Modesto KA, Martinez CBR. Roundup causes oxidative stress in liver and inhibits acetylcholinesterase in muscle and brain of the fish Prochilo dus lineatus. Chemosphere. 2010; 78: 294-299.

47. Glusczak L, Miron DS, Crestani M, Fonseca MB, Pedron FA, Duarte MF, et al. Effect of glyphosate herbicide on acetylcholinesterase activity and metabolic and hematological parameters in piava (*Leporinus obtusidens*). Ecotoxicol Environ Saf. 2006; 65: 237-241.

48. Barcellos LJG, Kreutz LC, Rodrigues LB, Fioreze I, Quevedo RM, Cericato L, et al. Haematological and biochemical characteristics of male jundiá (Rhamdia Quelen, Quoy & GaimaRDT, Pimelodidae): changes after acute stress. Aquacul. Res. 2003; 34: 1465-1469.

49. Benedetti AL, Vituri C de L, Trentin AG, Domingues MA, Alvarez-Silva M. The effects of sub-chronic exposure of Wistar rats to the herbicide Glyphosate-Biocarb. Toxicol Lett. 2004; 153(2): 227-232.

50. Glusczak L, Miron DS, Moraes BS, Simoes RR, Schetinger MRC, Morsch VM, et al. Acute effects of glyphosate herbicide on metabolic and enzymatic parameters of silver catfish (*Rhamdia quelen*). Comp Biochem Physiol. 2004; 146C: 519-524.

51. Harish RS, Murugan K. Oxidative stress indices in natural populations of Avicennia alba Blume as biomarker of environmental pollution. Environ Res. 2011; 11(8): 1070-1073.

52. Hazarika A, Sarkar SN, Hajare S, Kataria M, Malik JK. Influence of mala thion pretreatment on the toxicity of anilofos in male rats: a biochemical interaction study. Toxicol. 2003; 185: 1-8.

53. Hietanen E, Linnainmaa K, Vainio H. Effects of phenoxyherbicides and glyphosate on the hepatic and intestinal biotransformation activities in the rat. Acta Pharmacol Toxicol. 1983; 53(2): 103-112.

54. Howe CM, Berrill M, Pauli DB, Helbing CC, Werr K, Veldhoen N. Toxicity of glyphosate-based pesticides to four North American frog species. Environ Toxicol Chem. 2004; 23: 1928-1938.

55. Oluwafemi RA, Isiaka O, Alagbe JO. Recent trends in the utilization of medicinal plants as growth promoters in poultry nutrition- A review. Res Agric Vet Sci. 2020; 4(1): 5-11.

56. Olatunji AK, Alagbe JO, Hammed MA. Effects of varying levels of Moringa olifera leaf meal on performance and blood profile of weaner rabbits. Int J Sci Res. 2016; 5(6): 803-806.

57. Busari OO, Shittu MD, Bamise EI, Alagbe JO, Omowumi F. Gut nutrient absorptive potential and blood profile of Isa-brown laying birds fed varying inclusion levels of *Sida acuta* leaf meal. J Food Nutr. 2026; 5(1): 43.

58. Alagbe JO, Anorue DN. Influence of fermented Hunteria umbellata seed oil on the haematology, serum biochemistry, immune response and oxidative status of Growing Rabbits. Clin Sci Clin Res. 2026; 8(1): 16.

59. Alagbe JO. Haemato-biochemical indices of growing rabbits fed diets containing *Hunteria umbellata* seed meal as replacement for Soybean meal. Hematol Disord. 2026; 5(1): 18.

60. Emmanuel AU, Olafadehan OA, Ijeoma OC, Alagbe JO. Seminal morphology and organ morphometrics of rabbit bucks fed *Piliostigma thonningii* essential oil supplemented diet. Sci Lett. 2024; 12(2): 70-75.